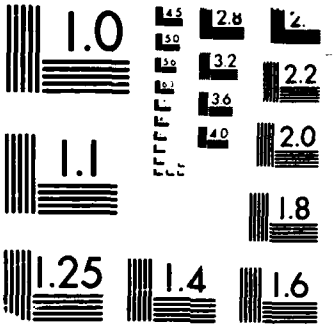
AD-A194 865 LOW DOSES OF ATROPINE SULFATE IMPAIR RETENTION OF A WELL-LEARNED SPATIAL TASK(U) ARMY RESEARCH INST OF ENVIRONMENTAL MEDICINE NATICK MA T M BAUCH ET AL. 1/1

UNCLASSIFIED APR 88 F/G 5/8 NL



UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

DTIC FILE COPY (1)

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

AD-A194 065

DTIC ELECTE MAY 03 1988 D

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: US Army Research Institute of Environmental Medicine	6b. OFFICE SYMBOL (If applicable): SGRD-UE-HP
7a. NAME OF MONITORING ORGANIZATION: US Army Medical Research & Development Command	
6c. ADDRESS (City, State, and ZIP Code): Natick, MA 01760-5007	7b. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701-5012
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: Same as 6a.	8b. OFFICE SYMBOL (If applicable)
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State, and ZIP Code): Same as 6c.	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62787A	3E1627-87A879	BE	DA 311337

11. TITLE (Include Security Classification)
Low Doses of Atropine Sulfate Impair Retention of a Well-Learned Spatial Task

12. PERSONAL AUTHOR(S)
T.M. Rauch, D.I. Welch and L. Gallego

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Manuscript	FROM _____ TO _____	April 88	22

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	atropine sulfate, dose-response, spatial memory

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Retention of a well-learned spatial task was assessed in rats 10 minutes prior to, and 10, 20, 30, 40 and 50 minutes after treatment with 3, 10 or 30 mg/kg, iv, atropine sulfate or the equivalent volume of saline, iv. There was a variable dose effect for escape latency and choice accuracy measures of spatial retention. A relatively large dose of atropine sulfate (30 mg/kg, iv) significantly impaired choice accuracy and escape latency compared with the control group. Moreover, impairment in choice accuracy was observed with smaller doses of atropine sulfate (3, 10 mg/kg, iv) than have previously been shown to disrupt spatial retention.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Terry M. Rauch	(617) 651-4195	SGRD-UE-HP

DD Form 1473, JUN 86 — Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

LOW DOSES OF ATROPINE SULFATE IMPAIR RETENTION OF A WELL-LEARNED SPATIAL TASK

T.M. Rauch, D.I. Welch and L. Gallego

U.S. Army Research Institute of Environmental Medicine
Natick, MA 01760

Running heading: Atropine Impairs Spatial Retention

SUMMARY

Retention of a well-learned spatial task was assessed in rats 10 minutes prior to, and 10, 20, 30, 40 and 50 minutes after treatment with 3, 10 or 30 mg/kg, iv, atropine sulfate or the equivalent volume of saline, iv. There was a variable dose effect for escape latency and choice accuracy measures of spatial retention. A relatively large dose of atropine sulfate (30 mg/kg, iv) significantly impaired choice accuracy and escape latency compared with the control group. Moreover, impairment in choice accuracy was observed with smaller doses of atropine sulfate (3, 10 mg/kg, iv) than have previously been shown to disrupt spatial retention.

Acknowledgements

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation.

Address communications to: Dr. Terry M. Rauch, Director, Health and Performance Division, U.S. Army Research Institute of Environmental Medicine, Kansas Street, Natick, MA 01760

INTRODUCTION

There is strong evidence that the hippocampus constructs and stores cognitive maps of spatial relationships (O'Keefe and Nadel, 1978; Olton, 1978; Olton, Becker and Handelmann, 1979; Sutherland, Whishaw and Kolb, 1982; Ellen and Wages, 1984; Ellen and Weston, 1983). In addition, numerous studies indicate that the septo-hippocampal pathway is cholinergic (Chippendale, Cotman, Kozar and Lynch, 1974; Lewis and Shute, 1967; Herink, Bajgar and Patocka, 1975; Fibiger, 1982; Johnson, McKinney, & Coyle, 1979; Lehman, Nagy, Atmadja, & Fibiger, 1980). Comparable evidence points to the pronounced similarities between impairments in spatial performance produced by cholinergic antagonists and those of hippocampal lesions (O'Keefe and Nadel, 1978). O'Keffe and Nadel (1978) suggest that the septo-hippocampal system plays a central role in synchronizing and controlling phase relations of various hippocampal neuronal fields involved in the formation and utilization of spatial maps during "navigation." Disruption of cholinergic synapses in this area produces an inability to utilize spatial mapping strategies to process spatial information.

One type of spatial performance, known as place navigation, requires a rat to learn the location of a spatially fixed platform hidden in a water maze. Accurate navigation is rewarded by escape from the water onto the platform. Most studies to date, have investigated the effects of anticholinergic agents, specifically atropine sulfate, on the acquisition of a spatial task. Sutherland, Whishaw & Regehr (1982) reported that atropine sulfate (50 mg/kg, ip), impaired the acquisition of a place navigation task compared to atropine methylnitrate. Atropine methylnitrate was used as a periperal control since it does not readily penetrate the brain (Albanus, Hammarstrom, Sundwall, and Ulberg, 1968). Despite the impairment, however, the atropine sulfate treated rats improved their spatial performance throughout the experiment. Sutherland et al., (1982) further reported that the atropine sulfate treatments did not impair motor activities, such as swim speed and pattern, involved in the assessment of spatial performance. Hagan, Tweedie and Morris (1986) showed that atropine sulfate (50 mg/kg, ip), administered to rats 30 minutes prior to training, impaired learning of both spatial and nonspatial discrimination tasks, compared to groups treated with saline or atropine methylnitrate (50 mg/kg, ip). In the same study, atropine sulfate (50 mg/kg, ip) also impaired spatial discrimination accuracy (but not escape latency), in rats previously trained to asymptote under drug-free conditions. Hagan et al., (1986) concluded that these

impairments were neither due to peripheral drug effect, since there were no significant differences in the spatial performance of saline and atropine methylnitrate treated animals, nor to gross sensorimotor impairments.

Large doses of atropine sulfate (50-100 mg/kg,), administered by intraperitoneal injection, have typically been used to block central cholinergic function and impair spatial performance (Whishaw, 1985; Hagan, Tweedie and Morris, 1986; Ellen, Taylor and Wages, 1986; Sutherland, Whishaw and Regehr, 1982; Whishaw and Tomie, 1987). This dose range is typically selected because it alters forebrain acetylcholine release (Dudar, Whishaw and Szerb, 1979), forebrain electroencephalographic activity (Vanderwolf, 1975) and produces virtually complete occupation of muscarinic binding sites in the rat brain (Yamamura, Kuhar and Snyder, 1974). In a recent study, however, we reported the differential sensitivity of two measures of spatial retention, choice accuracy and escape latency, to equal doses of atropine sulfate (30 mg/kg) administered by intravenous (iv), intraperitoneal (ip) and subcutaneous (sc) injection (Rauch et al., Note 1). The iv and ip atropine sulfate treatments produced a significant impairment in choice accuracy while only iv atropine sulfate resulted in a significant impairment in escape latency. Treatment with atropine sulfate sc never produced significant impairments in spatial retention when compared to the control group. The differences among iv, sc and ip treatments most likely reflect patterns of absorption, distribution and biotransformation for each site of injection.

Aropine sulfate (50-75 mg/kg, ip) in large doses has also been reported to elicit atropine stereotypy which prevents the animal from using more adaptive behaviors to escape from a partial enclosure (Schallert, De Ryck and Teitelbaum, 1980). Schallert et al., (1980) reported that in atropinized animals "behavioral traps" are reflexive reactions to one stimulus which leads it to encounter another, the reaction to which leads it to reencounter the first. We recently found that three out of six animals treated with iv atropine sulfate (30 mg/kg) and two of six treated with ip atropine sulfate (30 mg/kg) exhibited atropine stereotypy when tested on a spatial retention task (Rauch, Welch and Gallego, Note 1). There were no observations of stereotypy, however, among rats treated with the same dose of atropine (30 mg/kg) by sc injection. We attributed the incidence of stereotypy to differences in patterns of absorption, distribution and biotransformation among iv, ip and sc atropine sulfate treatments.

With the exception of Hagen et al., (1986) and Rauch et al., (Note 1), no studies have investigated the effects of central cholinergic blockade, with atropine sulfate, on the retention of a spatial task. Moreover, ability to retain spatial information when a wide range of atropine sulfate doses are administered has not been investigated. We therefore sought to determine if the retention of a well-learned spatial task would be differentially sensitive to a broad dose range of atropine sulfate.

MATERIALS AND METHODS

Subjects. Forty eight experimentally naive adult male Charles River CD strain rats, aged 140 days (300-500g) at the time of testing served as subjects. All rats were housed individually in hanging wire mesh cages with ad-lib access to food and water. Behavioral testing was conducted between 0900 and 1500 hours.

Apparatus. The maze used for behavioral testing consisted of a central runway with three transverse arms (see Figure 1). The central runway was 300 cm in length and trisected with transverse arms 105 cm in length. The mid-line of each transverse arm was equally spaced along the central runway 75 cm apart. Hence, the mid-line of each transverse arm was located at 75 cm, 150 cm and 225 cm along the length of the central runway. The first 30 cm at each end of the central runway served as a start alley. The width of the runway and each transverse arm was 21 cm and the height was 70 cm. The maze was placed in a large rectangular animal watering tank 325 cm in length and 150 cm in width. The interior of the tank and all sides of the maze were flat white. Prior to testing, the maze was filled to a depth of 37 cm with water (26 $\pm$ 1° C) and rendered opaque by adding 1 kg of dried powdered milk. A square escape platform, 10 cm by 10 cm, was constructed of white plastic and weighted at the base. The escape platform was placed at the A or B position (see Figure 1) of the middle transverse arm 4 cm below water level. After each day of testing the tank was drained and cleaned. The maze was illuminated by overhead lights and the testing room contained numerous extramaze cues such as a laboratory table, sink and chair.

Spatial Learning Procedure. Prior to the first week of training the animals were handled each day for approximately three minutes. One day before training each animal was placed in the open water tank with no escape platform and allowed to swim for 90 s. The animals were then

returned to their home cages. On the two training days animals were trained in a fixed order, ten trials in the morning and ten trials in the afternoon for two days. For each animal, across trials, the position of the start alley was randomized but the submerged escape platform was always fixed at the same end of the middle transverse arm. However, the position of the escape platform was randomized across animals. A trial started with the rat held facing away from the center of the tank, immersed in water, and at the end of the start alley. Each animal was allowed 120 s to escape onto the platform. If the animal failed to escape within this time it was guided to the platform. Whether or not the animal had escaped or been guided to the platform, it remained there for 15 s before being removed and placed in the home cage for a 10 min intertrial interval. On each trial first choice accuracy (platform always correct) and escape latency were recorded. A choice error was recorded when the animal's head entered a non-platform arm.

Atropine Administration and Spatial Retention Procedure. On drug days each animal was randomly assigned to one of four groups: 3, 10 or 30 mg/kg atropine sulfate (Sigma Chemical Co., St. Louis, MO) dissolved in 0.2 ml of sterile saline or 0.2 ml of sterile saline used as the control. A 30 mg/kg, iv dose was chosen since we found this dose (iv) to disrupt first choice accuracy and escape latency measures of spatial retention (Rauch et al., Note 1). Ten minutes prior to injection with atropine sulfate or saline, each animal was tested for one trial on their retention of the spatial task, then injected and returned to their home cage. All injections were made iv into a lateral tail vein. Animals were tested over five trials at 10, 20, 30, 40, and 50 minutes post injection. Hence, spatial retention was assessed over six trials, one immediately prior to injection, and five trials at ten minute intervals after injection.

RESULTS

The analysis was performed on the number of animals making a correct first choice (choice accuracy) and on escape latency. All animals rapidly learned to swim to the hidden escape platform prior to treatment with atropine sulfate. Over 40 trials the mean escape latency declined from 68 to 5 seconds. In addition, 92% of the animals made a correct first choice to the escape platform over trials 35 to 40. There was a significant effect of trials on choice accuracy, F (39,1716) = 22.44, $p < .001$ and on escape latency, F (39,1716) = 29.84,

$p < .001$. Neither the dose effect ($F < 1$) nor the dose X trials interaction ($F < 1$) was significant. These data are summarized in Figure 2 and show that performance increased up to 25 trials and approached asymptote the last ten trials.

Treatment with atropine sulfate produced significant impairments in both measures of spatial retention. The effects of different doses of atropine sulfate on escape latency over six trials are shown in Figure 3. There was a significant main effect for dose, F (3,44) = 4.28, $p < .01$, and a significant dose X trials interaction, F (15,220) = 1.78, $p < .05$, on escape latency. There was no significant main effect of trials. Post hoc Tukey ($p < .05$) comparisions of group means for each trial revealed a significant impairment in escape latency for the 30 mg/kg atropine sulfate dose compared with saline at 10 and 20 minutes post injection.

The overall accuracy of performance is shown in Figure 3, where the number of correct 1st choices are plotted for different doses of atropine sulfate over six trials. There was a significant main effect of dose, F (3,44) = 4.15, $p < .02$, and trials, F (5,220) = 10.30, $p < .001$, on choice accuracy. Moreover, there was a significant dose X trials interaction, F (15,220) = 2.08, $p < .02$. Post hoc comparisons of group means for each level of dose and trial revealed that saline treated animals made significantly more correct 1st choices compared to animals treated with 30 and 10 mg/kg atropine sulfate at 20 minutes post injection. Saline treated animals also made significantly more correct 1st choices than the 3, 10 and 30 mg/kg atropine sulfate treated animals, at the 30 minute trial. Post hoc comparisons of group means for different levels of dose over six trials revealed significantly poorer choice accuracy for the 30 mg/kg group at 10, 20, 30 and 40 minutes post injection compared with the pre injection trial. However, choice accuracy for the 30 mg/kg group at 50 minutes post injection was significantly greater than the 10 and 20 minute post injection trials. Choice accuracy for the 3 mg/kg group was significantly poorer at 10, 20 and 30 minutes post injection when compared to the pre injection trial, while for the 10 mg/kg group choice accuracy was significantly poorer at 20 minutes post injection when compared to the pre injection trial.

DISCUSSION

This study demonstrates that a broad dose range of atropine sulfate disrupts the retention of a well-learned spatial task. The present data support our findings (Rauch et al., Note 1) and those of

Hagen et al. (1986), that a relatively large dose of atropine sulfate (30 mg/kg, iv and 50 mg/kg, ip, respectively) impairs choice accuracy in a spatial retention task. The present results also show that escape latency for the 30 mg/kg, iv, atropine sulfate treated rats was significantly impaired. A significant disruption in choice accuracy was achieved with smaller doses of atropine sulfate (3, 10 mg/kg, iv) than have previously been found to impair that measure of spatial retention. Since the behavioral effects of atropine sulfate were demonstrated on a different spatial task than the Morris water maze (Morris, 1981), the present results extend the range of behavioral tasks that demonstrate the role of central cholinergic mechanisms in reference memory and place navigation.

The choice accuracy measurement proved to be an extremly sensitive measure of spatial performance. Pretreatment with the lowest dose of atropine sulfate (3 mg/kg, iv) and the intermediate (10 mg/kg, iv) dose reduced the number of animals making a correct first choice to approximately 45% of the control (i.e., saline) value at 30 minutes post injection. Moreover, the maximal dose of atropine sulfate reduced choice accuracy to 27% of the control level (see Figure 3). The impairment reported for the 3 mg/kg group suggests that minimal cholinergic blockade is required to disrupt choice accuracy. Hagen et al., (1986) showed impaired choice accuracy in spatial retention in rats administered a 50 mg/kg, ip dose. The present data also indicate over a four-fold increase in the escape latency of the 30 mg/kg group over control values at 10 and 20 minutes post injection (see Figure 3). The initial impairment and subsequent improvement in choice accuracy for the 30 mg/kg, iv, atropine sulfate group across trials may be the result of re-learning the place response. Whishaw (1985) reported that atropine sulfate treated rats can acquire a place response, but at a significantly slower rate than controls. Our results from a recent study (Rauch et al., Note 1) show that atropinized rats improve their choice accuracy over subsequent trials at 10, 20, 30, 40 and 50 minutes after injection.

REFERENCE NOTES

1. Rauch TM, Welch DI Gallego L (1988): Retention of a spatial task after intraperitoneal, subcutaneous or intravenous injections of equal doses of atropine. Manuscript submitted for publication

REFERENCES

Albanus L, Hammarstrom L, Sundwall A, Ulberg S, Vango B (1968): Distribution and metabolism of ^{3}H-atropine in mice. Acta Physiol Scand 73: 447-456

Chippendale TJ, Cotman CW, Kozar MD, Lynch GS (1974): Analysis of acetylcholinerterase synthesis and transport in the rat hippocampus: Recovery of acetylcholinesterase acitvity in the septum and hippocampus after administration of diisopropylfluorophosphate, Brain Res 81: 485-496

Dudar JD, Whishaw IQ, Szerb JC: (1979) Release of acetylcholine for the hippocampus of freely moving rats during sensory stimulation and running. Neuropharmacology 18: 673-678

Eckerman DA, Gordon WA, Edwards JD, MacPhail RC, Gage MI (1980): Effects of scopolamine, pentobarbital and amphetamine on radial arm maze performance in the rat. Pharmacol Biochem and Behav 12: 595-602

Ellen P, Weston SL (1983): Problem solving in the rat: Septal lesion effects on havituation and perserverative tendencies. Physiol Psych 11: 112-118

Ellen P, Wages C (1984): The use of intramaze stimuli in the attenuation of the problem-solving deficit of septal animals. Physiol Psych 12: 133-140

Ellen P, Taylor H.S, Wages, C (1986): Cholinergic blockade effects on spatial integration versus cue discrimination performance. Behav Neurosci 100:720-728

Fibiger HC (1982): The organization and some projections of cholinergic neurons of the mammalian forebrain. Brain Rev 4:327-388

Hagan JJ, Tweedie F, Morris RGM (1986): Lack of task specificity and absence of posttraining effects of atropine on learning. Behav Neurosci 100: 483-493

Herink J, Bajgar J, Patocka J (1975): Changes of acetylcholinesterase activity is some parts of the limbic system following septal lesions in rats. J Neurochem 24: 187-188

Johnson M, McKinney M, Coyle J (1979): Evidence for a cholinergic projection to neocortex from neurons in basal forebrain. Proc Natl Acad Sci (USA) 76: 5392-5396

Lehman J, Nagy JI, Atmadja S, Fibiger HC (1980): The nucleus basalis magnocellularis: The orgin of a cholinergic projection to the neocortex of the rat. Neurosci 5:1161-1174

Levy A, Kluge PB, Elsmore TE (1983): Radial arm maze performance of mice: Acquisition and atropine effects. Behav Neurl Biol 39:229-240

Lewis PR, Shute, CCD (1967): The cholinergic limbic system. Projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system and the subfornical organ and supra-optic crest. Brain 90: 521-540

Morris RGM (1981): Spatial localization does not require the presence of local cues. Learning and Motivation 12: 239-261

O'Keefe J, Nadel L (1978): The hippocampus as a cognitive map. Oxford: Oxford University Press

Olton DS (1978): The function of septo-hippocampal connections in spatially-organized behavior. In: Functions of the septo-hippocampal system. CIBA Foundation Symposium 58. Elsevier Amsterdam (pp 327- 342)

Olton DS, Becker JT, Handelmann GE (1979): Hippocampus, space and memory. Behav Brain Sci 2: 313-365 (includes commentary)

Sutherland RJ, Whishaw IQ, Kolb B (1982): A behavioral analysis of spatial localization following electrolytic, kainate- or colchicine-induced damage to the hippocampal formation in the rat. Behav Brain Res 7: 133-153

Schallert T, DeRyck M, Teitelbaum P (1980): Atropine sterotypy as a behavioral trap: A movement subsystem and electroencephalographic analysis. J Comp Physiol Psychol 94: 1-24

Sutherland RJ, Whishaw IQ, Regehr JC (1982): Cholinergic receptor blockade impairs spatial localization of use of distal cues in the rat. J Comp Psysiol Psychol 96: 563-573

Vanderwolf CH (1975): Neocortical and hippocampal activation in relation to behavior: Effects of atropine eserine, phenothiazines, and amphetamine. J Comp Physiol Psychol 88: 300-323

Vanderwolf CH, Robinson TE (1981): Reticulo-cortical activity and behavior: A critique of the arousal theory and a new synthesis. Behav Brain Sci 4: 459-514

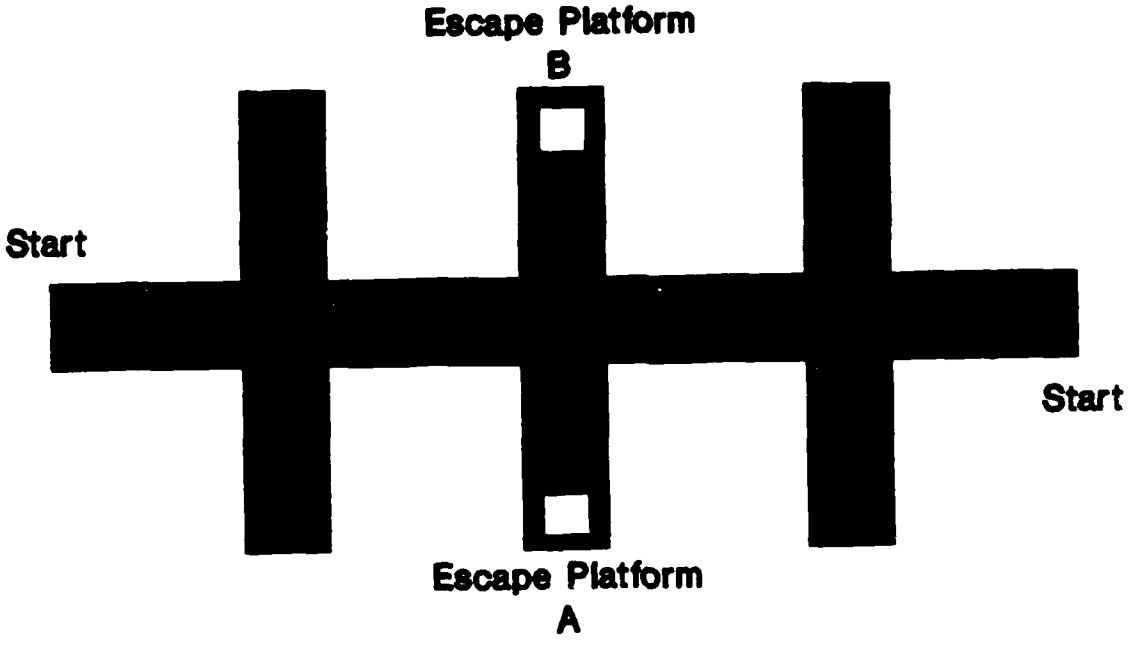
Whishaw IQ (1985): Cholinergic receptor blockade in the rat impairs locale but not taxon strategies for place navigation in a swimming pool. Behav Neurosci 99: 979-1005

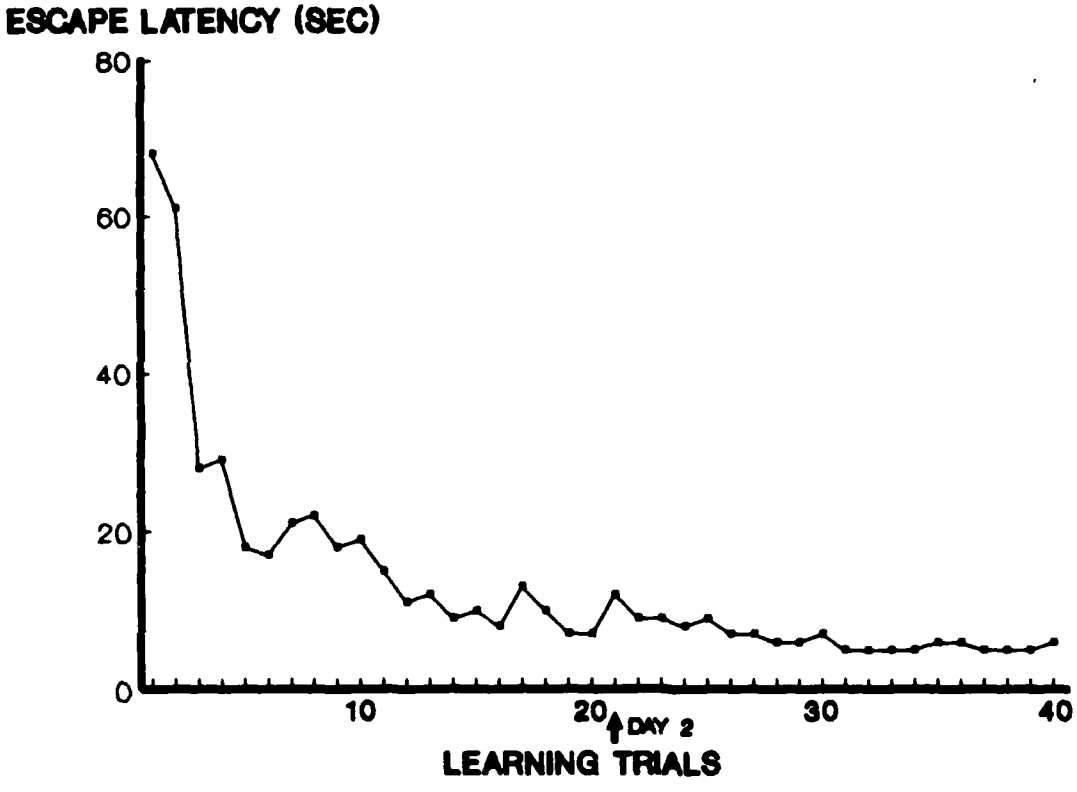
Yamamura HI, Kuhar MJ, Snyder SJ (1974): In vivo identification of muscarinic cholinergic receptor binding in rat brain. Brain Res 80: 170-176

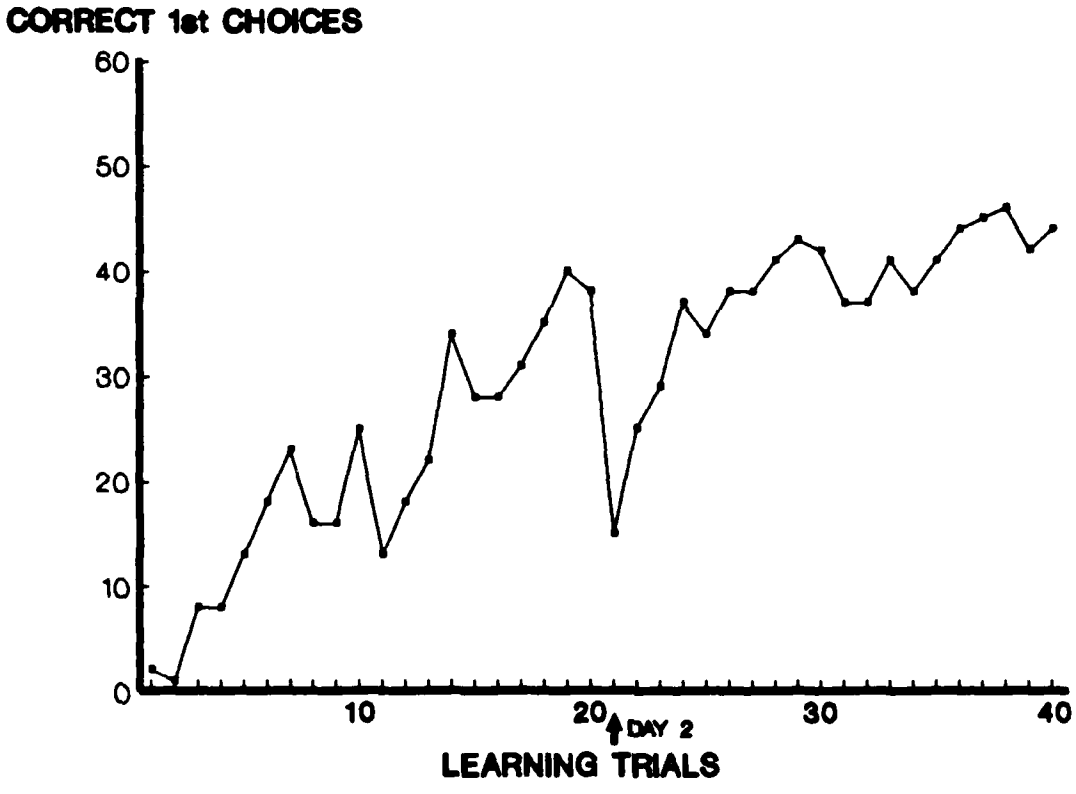
Figure 1. Modification of a multiple cross maze as diagrammed was used for acquisition and retention of a spatial task.

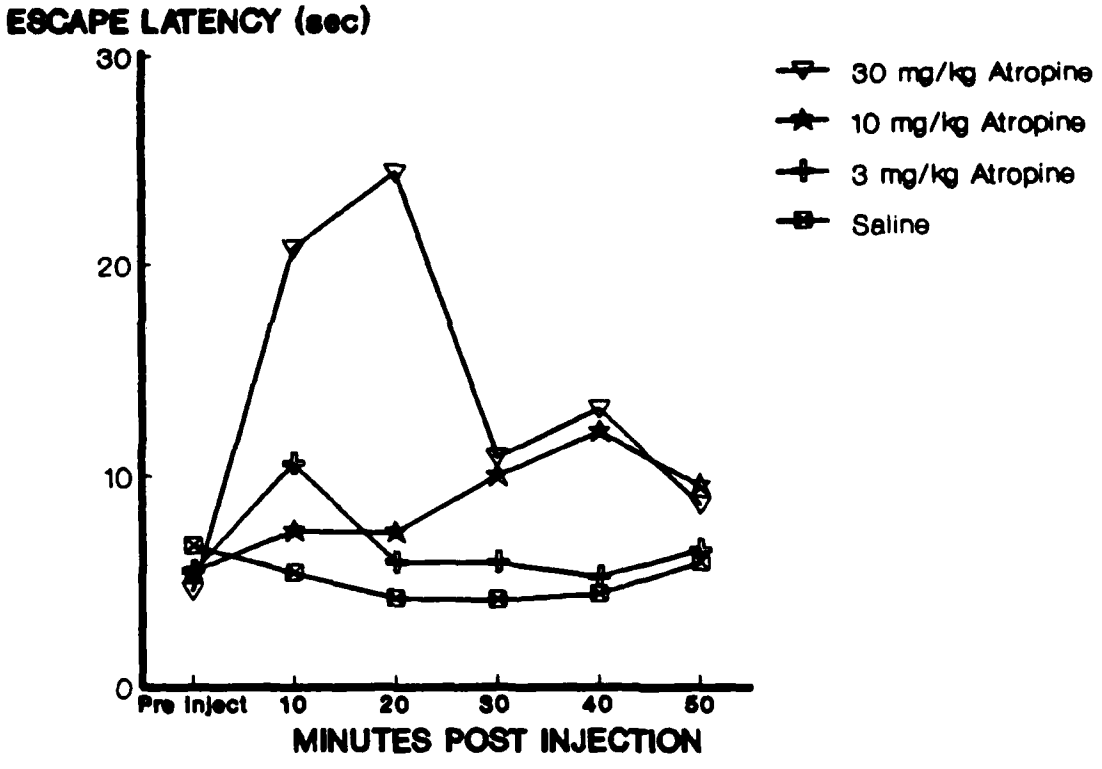
Figure 2. Mean escape latency (top) and number of correct first choices (bottom) on each trial for all animals during acquisition of the spatial task.

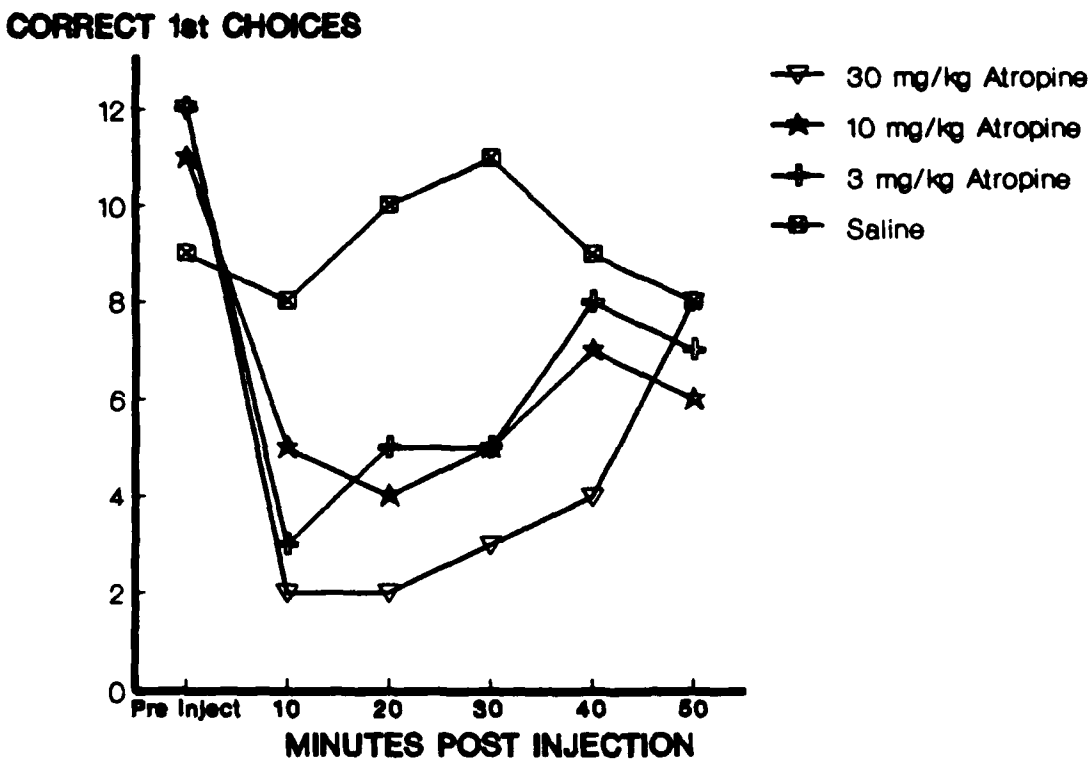
Figure 3. Retention of a spatial task: Mean escape latency (top) and number of correct first choices (bottom) immediately pre, and 10, 20, 30, 40 and 50 minutes, post injection of saline or variable doses of atropine sulfate.


Escape Platform
B
Start
Start
Escape Platform
A


ESCAPE LATENCY (SEC)
80
60
40
20
0
10
20
DAY 2
30
40
LEARNING TRIALS


CORRECT 1st CHOICES
60
50
40
30
20
10
0
10
20
DAY 2
30
40
LEARNING TRIALS


ESCAPE LATENCY (sec)
30
20
10
0
Pre Inject
10
20
30
40
50
MINUTES POST INJECTION
30 mg/kg Atropine
10 mg/kg Atropine
3 mg/kg Atropine
Saline


CORRECT 1st CHOICES
12
10
8
6
4
2
0
Pre Inject
10
20
30
40
50
MINUTES POST INJECTION
30 mg/kg Atropine
10 mg/kg Atropine
3 mg/kg Atropine
Saline

END
DATE
FILMED
7-88
DTIC